Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. (July–Sept 2018) 88(3):959–966
https://doi.org/10.1007/s40011-016-0833-9

RESEARCH ARTICLE

Phosphorus Solubilization and Plant Growth Promotion Ability of Rhizobacteria of *R. communis* L Growing in Assam, India

Sosanka Protim Sandilya[1] · Pinky Moni Bhuyan[1] · Dip Kumar Gogoi[1] · Devid Kardong[2]

Received: 19 July 2016 / Revised: 6 September 2016 / Accepted: 11 November 2016 / Published online: 22 November 2016

Abstract Castor (*Ricinus communis* L) is the primary host plant of eri silkworm and its rhizosphere harbours diverse group of microbial community with biofertilizer potentiality. Phosphate solubilizing bacteria (PSB) render available phosphate (P) in agricultural soil by P mineralization process through enzyme mediated reaction. In search for PSB strains, 15 castor rhizobacteria were isolated and characterized for morphological and biochemical properties. The isolates were screened in vitro for P solubilization efficiency both qualitatively and quantitatively. Isolate MAJ PSB12 produced highest soluble P concentration (322.20 µmol/l) in National Botanical Research Institute Phosphate medium after 96 h of incubation with a maximum drop in pH to 5.4 from 7.0. Among the isolates, maximum content of IAA (24.6 mg/l) and GA_3 (3.921 mg/l) was also found to be produced by the same strain. The most potential isolate was identified as *Bacillus firmus* MAJ PSB12 by 16S rRNA gene homology analysis and the sequence was submitted to National Centre for Biotechnology Information GenBank. Although many species belonging to the genus *Bacillus* are efficient P solubilizer, application of native rhizobacteria is easier for adaptation and succession during biofertilization process. *B. firmus* MAJ PSB12 can be utilized as potential biofertilizer to promote sustainable castor cultivation in sericulture for upliftment of rural livelihood.

✉ Dip Kumar Gogoi
gkdeep@rediffmail.com

[1] Biotechnology Section, CMER&TI, Central Silk Board, Lahdoigarh, Jorhat, Assam, India

[2] Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India

Keywords Phosphate solubilizing bacteria · Plant growth promoting rhizobacteria · Gene homology · *Bacillus firmus* · Sericulture

Introduction

Castor (*Ricinus communis* L), an annual or biennial herb belonging to the family Euphorbiaceae, is utilized as a primary host plant of Eri silkworm (*Samia ricini* Donovan) since time immemorial. *S. ricini* endemic to Northeast India is a multivoltine sericigenous insect that produces Eri silk and has been a good source of rural livelihood. Here, the rate of production stream lies specifically in the proper consumption of the quality leaves of the host plant.

The quantity and nutritional quality of castor leaf is dependent on soil fertility. Phosphorus is the most important limiting factors for growth and development of plants just after nitrogen [1] and plays a decisive role in the physiological processes of a plant body. Many sericulture farmers of Northeast India mostly opt for adequate inorganic phosphorus fertilizer, which is considered a major source of heavy metal contamination in agricultural soils [2, 3] and causes eutrophication of surface and groundwater sources [4, 5]. In spite of low concentration and slow rate of nutrient release as compared to synthetic fertilizers, biofertilizers offer an eco-friendly, cost-effective and sustainable alternative to chemical fertilizers [6–9].

Phosphorus mobilizing bacteria are a group of microorganisms that produce phosphatase enzymes and mobilize the insoluble form of phosphate, for direct nutritional uptake by plants to reduce the utilization of agrochemicals [10]. Phosphate solubilization is a complex phenomenon and it depends upon nutritional, physiological and growth conditions of the microorganisms inhabited in

the rhizosphere [11]. Many of the bacterial and fungal genera like *Azospirillum, Azotobacter*, *Pseudomonas, Bacillus, Aspergillus,* etc. have been reported earlier to have growth promoting ability, reduction of nitrate and solubilization of phosphate as well [12].

Organic acids released by rhizobacteria potentially involve in different kind of soil reactions which include P mobilization and cation uptake by plant root system. Phosphorus mobilization efficiency in these processes depends on the amount and the type of organic acids released as well as on the physico-chemical properties of soils [13]. Most of the soils around the globe lack available P because it is easily converted into insoluble complexes such as iron and aluminium hydrous oxides and calcium phosphate complexes. Majority of organic acid secreting bacteria are gram negative primarily belong to Proteobacteria. Although, few genera of gram positive bacteria belongs to Firmicutes (e.g. *Bacillus*, *Paenibacillus*) and Actinobacteria (*Arthrobacter*, *Rhodococcus*, *Micrococcus*) are also reported to secrete organic acid [14].

There are very sporadic reports on the exploitation of beneficial rhizospheric microorganisms as biofertilizer to curtail the application of chemical fertilizers in sericulture sector. Thus, it is an important aspect to introduce potential strains which may help in redesigning the soil structure and enhancement of the soil fertility [15].

In this study, an effort has been made to isolate, characterize, screening for phosphatase activity and identification of the most potential phosphate solubilizing bacteria (PSB) from Castor rhizosphere soil of different localities of Assam for subsequent formulation of an INM package in sustainable Ericulture.

Material and Methods

Collection of Soil Samples

Castor rhizosphere soil samples were collected from various localities of Assam, foothills of Nagaland and Arunachal Pradesh, India. The samples were brought aseptically to the laboratory in sterile poly-bags, stored at 4 °C and processed within 24 h for further analysis [16].

Isolation and Enumeration of the Soil Bacteria

Phosphate solubilizing bacteria (PSB) were isolated by serial dilution spread plate technique using Pikovskaya's Agar media. Ten gram (10 g) of soil sample was suspended in 90 ml of sterile distilled water and serial dilutions were prepared upto 10^{-7}. Aliquots from the dilutions were inoculated and the plates were incubated (OVFU, O-CIS-4D) aerobically at 32 °C for 96 h. After incubation the phosphate solubilizing microorganisms were selected based on the zone of clearing around the colonies and purified by repeated culturing. The purified PSB were transferred to nutrient agar (NA) slant for further maintenance at 4 °C. Enumeration of the microbial population was done by total viable colony count method and expressed as number of colony forming units (CFU) in 1 ml of the soil sample.

Morphological and Biochemical Characterization

Cultural characters of the pure isolates were studied on the basis of colony elevation, margin, form, texture and opacity [17]. The phenotypic morphological study was conducted by Gram staining microscopy method using Gram staining kit (Hi-media K001) as per the method described by Holt et al. [18]. The stained bacterial cells were observed under phase contrast microscope (Olympus, CKX41) and the Gram reaction, shape, orientation etc for the efficient PSB strains were recorded.

Biochemical characterization was carried out by test for citrate utilization, phosphatase, urease, nitrate reduction, glucose, arabinose, maltose, starch, Voges proskaur, ONPG, catalase, arginine, sucrose, methyl red, gelatin liquefaction, indole, H_2S production, glycerol, trehalose, xylose, adonitol, galactose, gluconate, lactose, melibiose, rhamnose, ribose, sorbitol, xylitol and mannitol using biochemical test kit (Hi-media Ltd., Mumbai, India) and as per the Bergey's Manual of Determinative Bacteriology.

Screening for Phosphatase Activity

Qualitative screening of the PSB isolates was carried out by spot inoculation in petri-plates containing National Botanical Research Institute's Phosphate (NBRIP) media (Glucose 10 g/l, $Ca_3(PO_4)_2$ 5 g/l, $MgCl_2.\ 6H_2O$ 5 g/l, $MgSO_4.\ 7H_2O$ 0.25 g/l, KCl 0.2 g/l, $(NH_4)_2SO_4$ 0.1 g/l, Agar 15 g/l and pH adjusted to 7.0). The inoculated plates were incubated at 32 °C for 48–96 h under aerobic condition. The resultant formation of Halozone diameter around the single colonies was measured and recorded. Further, the solubilization index {the ratio of the total diameter (colony + halozone) and the colony diameter} was calculated [19, 20]. The potential isolates were thereafter selected for the quantitative estimation of available phosphate.

Quantitative Estimation

Estimation of available phosphate was carried out by Vanadomolybdate colorimetric assay method [21]. The efficient PSB isolates capable of producing phosphate solubilizing zone (PSZ) were further inoculated on

Nutrient broth (NB) medium and incubated at 30 °C in rotary shaker (100 rpm) to prepare the seed culture. Seed culture of PSB isolates (1×10^8 CFU/ml) was then transferred into a 250 ml flask in 3 replications per strain containing sterilized liquid NBRIP medium (100 ml) and incubated for different time intervals like 24, 48, 72 and 96 h with continuous shaking (REMI CIS-24 plus) at 30 °C. Sterilized un-inoculated NBRIP medium was treated as a control and inoculation with *Bacillus subtilis* NCIM2063 served as positive control. The resultant pH drop in the medium was recorded regularly at different time intervals with pH meter (Eutech Cyberscan) and cell numbers were estimated by standard plate count method [22]. Ten ml aliquot of each culture and control were taken after incubation and centrifuged at 10,000 rpm at 4 °C for 15 min and the clear supernatant was used to determine the amount of phosphorus released into the medium. The supernatant was incubated at 32 °C for 30 min and 5 ml of each culture was transferred to test tube. Finally, 2 ml of vanadomolybdate reagent was added to each tube and the colour developed was measured spectrophotometrically (Systronics 2202) at a wavelength of 420 nm. The available phosphorus was estimated by calibrating with standard KH_2PO_4 curve. Each treatment was replicated thrice and data were expressed as the mean value ± standard error.

IAA and GA_3 Activity

Indole acetic acid (IAA) produced by PSB isolates was estimated using tryptophan as per standard method [23, 24]. The bacteria were cultured overnight on nutrient broth and cells were collected by centrifugation at 14,000 rpm for 5 min. Cell pellets were suspended in 3 ml of phosphate buffer (pH 7.5) containing glucose (1%) and tryptophan (1%) and incubated at 37 °C for 24 h. After incubation, 2 ml of 5% trichloroacetic acid and 1 ml of 0.5 M $CaCl_2$ were added and filtered through Whatman paper no.2. Filtrates (3 ml) were taken in test tubes and 2 ml of salper solution (2 ml of 0.5 M $FeCl_3$ and 98 ml 35% perchloric acid) was added and mixed thoroughly. Then the mixture was incubated for 30 min at room temperature (25–28 °C) in dark. The absorbance of the colour developed in the solutions was measured at 535 nm using spectrophotometer (Systronics 2202).

Production of gibberellic acid (GA_3) was determined by following standard methodology [25]. Three ml of filtrate obtained by the above method for IAA test was taken in test tube and 2 ml of zinc acetate was added. After 2 min, 2 ml of potassium ferrocyanide was added and the solution was centrifuged at 1000 rpm for 5 min. Five ml of 30% HCl was added to the supernatant (5 ml) and incubated at 20 °C for 2 h. The absorbance of the sample was measured at 254 nm using uv–Vis spectrophotometer including the control (treated with 5 % HCl).

The concentrations of IAA and GA_3 produced by the PSB isolates were calculated by calibrating with standard curve prepared by using graded concentrations of IAA and GA_3 (Hi-media Ltd.) and expressed as mg/l. The overall IAA and GA_3 productions of the PSB isolates were compared with a control reference strain *Bacillus subtilis* MTCC 441 procured from MTCC, Chandigarh.

Molecular Identification of the Potential PSB Isolate

Molecular identification of the most potential isolate was carried out by 16S rDNA sequencing and homology study. Isolation of DNA of the potential phosphate solubilizer strain was done by standard methodology [26] using Genomic DNA extraction kit manufactured by SRL Pvt. Ltd., Mumbai (India). The DNA purity and quantity were checked by spectrophotometer at 260 and 280 nm, respectively. PCR amplification of 16S rDNA was done with bacterial universal forward primer 16SF (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 16SR (5′-ACGGCTACCTTGTTACGACTT-3′) as described earlier [27]. The PCR for the 16S rRNA gene was performed with initial denaturation at 95 °C for 2 min followed by 35 cycles consisting of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, followed by a final extension step of 5 min at 72 °C. The PCR products were purified using a QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions. The partial sequencing of the 16S rDNA gene was carried out through the courtesy of DNA sequencing service, Merck Millipore (Bangalore GeNei^{TM}), Bangalore, India by using the same primer.

The partial 16S rDNA gene sequence homology was analyzed through online BLAST programme by aligning to the closest phylogeny with known taxonomic information available at NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree was constructed by the Neighbor Joining method [28] using CLUSTALW. The identified gene sequence was submitted to NCBI GenBank and accession number was obtained for further reference.

Statistical Analysis

The arithmetic mean of three independent replications was calculated and tested for standard deviation. Determination of correlation co-efficient at 0.01/0.05 significance levels among the microbial population (CFU/ml), pH and soluble P concentration was carried out by the Statistical Analysis System (SAS) with the help of statistical software programme 'SPSS version 16'.

Results and Discussion

Eri sericulture is although an age old tradition particularly in Northeastern part of India, modern studies in this field are still lagging behind. Phosphorus is one of the most imperative limiting factors of soil whether in organic or inorganic form [1]. In spite of knowing the potentiality of PSB in mobilization of phosphate and other inorganic nutrients, the use of chemical fertilizers has become an epidemic in sericulture sector. The extensive use of inorganic fertilizers has threatened the sericulture/agriculture sector by deteriorating the soil health of this region [29]. The present work attempted to access the potentialities of the PSB, isolated from rhizosphere of naturally growing castor varieties in this locality for subsequent exploitation as biofertilizer.

Isolation and Characterization of PSB

In the present study, 360 pure cultures of PSB were isolated from 30 castor rhizosphere samples collected from different localities of Assam, foothills of Nagaland and Arunachal Pradesh (India). Maximum population (1.2×10^3 CFU/ml) of PSB was recorded as total viable colony in the soil sample collected from Majuli and it has been followed by the samples collected from Kaziranga, Golaghat, Pasighat, Rowing, and Sadiya. Most of the PSB isolates were found to be Gram positive rod shaped of various sizes as given in Fig. 1a and arranged in different orientations (Table 1). However, the amplified rDNA of MAJ PSB12 with DNA ladder (StepUp ^{TM} 500 bp) is given in Fig. 1b and the phylogenetic tree-based on the 16S rDNA sequence indicating the position of strain MAJ PSB12 (using Neighbor Joining method) is given in Fig. 1c.

All the PSB isolates exhibited diverse biochemical properties (Table 1) during the analysis for the biochemical tests as per Bergey's Manual of Determinative Bacteriology and Biochemical Test Kit (Hi-media Ltd., Mumbai).

Substantial populations of aerobic and anaerobic PSB strains that have been reported from soil and plant rhizosphere [30], but occurrence of aerobic strains was more in submerged soil. Significantly, the PSB population was denser in rhizosphere in comparison to the non-rhizospheric soil [31]. It has also been reported that the population level of PSB in rhizosphere soil varies depending upon the plant, type of soil, season, climate, geographical location etc. [32].

Screening of PSB

Out of 360 PSB isolates, 15 strains produced considerably higher solubilization index, during *in-vitro* qualitative screening in Pikovskaya's agar medium. Isolate MAJ PSB12 produced maximum solubilization index of 1.89, 3.0, 3.35 and 3.66 at 24 h, 48 h, 72 h and 96 h of incubation, respectively. Besides, isolates MAJ PSB11, MAJ PSB06 and MAJ PSB08 also exhibited higher P solubilization activity in preliminary screening. Based on visual plate screening and subsequent solubilization index for

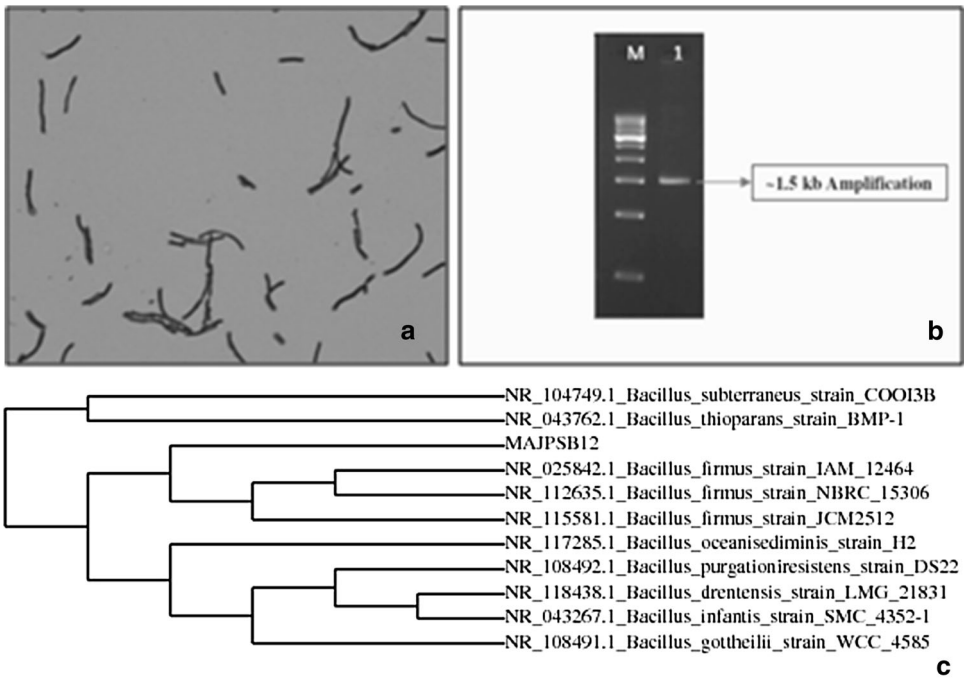

Fig. 1 *Bacillus firmus* MAJ PSB12: **a** microscopic photograph of *Bacillus firmus* MAJ PSB12; **b** amplified rDNA of MAJ PSB12 with DNA ladder (StepUp^{TM} 500 bp); **c** phylogenetic tree-based on the 16S rDNA sequence indicating the position of strain MAJ PSB12 (using Neighbor Joining method)

Table 1 Morphological and biochemical properties of isolate no. MAJ PSB12

Characteristic features	Result	Characteristic features	Result
Gram reaction	Positive	*Sugar utilization test*	
Morphology	Rod	Glucose	+
Motility	+	Sucrose	+
Starch hydrolysis	+	Mannitol	+
Gelatin liquefaction	+	Glycerol	+
Voges proskaur	−	Maltose	+
Citrate utilization	−	Trehalose	+
Indole production	+	Xylose	−
H_2S production	−	Arabinose	+
Urea hydrolysis	+	Galactose	−
Nitrate	+	Lactose	−
Phosphatase	+	Rhamnose	−
Catalase production	+	Ribose	−
Methyl red	+	Sorbitol	−
N-acetyl-D-Glucosamine	+	Xylitol	−
ONPG	−	Adonitol	−
β-galactosidase	−	Melibiose	−

phosphate solubilization ability, 15 PSB isolates were selected for further assay.

P Solubilization

Total viable cell densities in CFU/ml, pH and amount of soluble P in the NBRIP medium were determined at different time intervals. Result showed that the bacterial population and P concentration in broth culture increase with the incubation period up to 96 h. The soluble P concentration in the NBRIP medium after 96 h of incubation was estimated ranging between 322.20 ± 10.78 and 95.81 ± 10.67 µmol/l. Simultaneously, significant decrease of pH of the medium up to 5.4 from an initial pH of 7.0 was observed after 96 h. Out of 15 PSB isolates, isolate MAJ PSB12 showed highest P solubilization (322.20 ± 10.78 µmol/l) with maximum drop in pH to 5.4, followed by KAZ PSB03 with soluble P concentration 222.17 ± 10.44 µmol/l (Fig. 2). Minimum concentration of soluble P (95.81 ± 10.67 µmol/l) was recorded in MAJ PSB03 with culture medium pH 7 after 96 h of incubation at 30 °C. In the blank, i.e. un-inoculated medium, no soluble P was detected as well no pH change was recorded. On the contrary, in the *Bacillus subtilis* NCIM2063 (positive control) culture, the concentration of soluble P was 262.18 µmol/l and the pH dropped to 5.8. Statistically, the bacterial population and P solubilization in NBRIP medium are positively correlated whereas pH of culture broth is negatively correlated with soluble P concentration at different time intervals. Qualitative screening and quantitative assay of the PSB isolates revealed that isolate MAJ PSB 12 is the most efficient phosphate solubilizer which is considered for further species level identification. Although, maximum pH drop was directly proportional to the amount of soluble P concentration, but some isolates performed a considerable level of phosphate solubilization where the decrease of pH was negligible.

In-vitro phosphate solubilization dynamics of the isolates was carried out based on the measurement of P release into culture broth, from the cultures grown in medium containing insoluble P. In the present study, maximum production of soluble phosphate was recorded at 96 h of incubation with initial pH drop from 7 to 5.4. Phosphate solubilization by the PSB isolates is negatively correlated with pH, as it may be due to the production of various organic acids during solubilization process. The production of 8 different kinds of organic acids by HPLC detection method was reported [33]. Production of acids leads to acidification of the media by dropping the pH during P solubilization. Similar type of negative correlation was reported earlier by several workers [34]. On the contrary, some investigators [35–37] reveal that pH is not correlated with P solubilization particularly in case of *P. aurantiogriseum* [38] and *P. radicum* [39] over a period of 7 days of incubation.

IAA and GA_3 Production

Screening of results revealed that almost all the PSB isolates are capable of producing IAA and GA_3 (Fig. 3). Total content of IAA synthesized by the isolates ranged from 8.5 ± 0.66 mg/l to 24.6 ± 0.37 mg per liter of nutrient broth, whereas GA_3 production ranged from 0.123 ± 0.13 to 3.921 ± 0.17 mg/l. Maximum content of IAA (24.6 ± 0.37 mg/l) was produced by isolate MAJ PSB12 and lowest production (8.5 ± 0.66 mg/l) was recorded by isolate KAZ PSB03 at 96 h of incubation. Considerably higher amount of IAA was produced by isolates MAJ PSB 05 (21.19 ± 0.35 mg/l), MAJ PSB 03 (20.39 ± 0.48 mg/l) and KAZ PSB 01(18.80 ± 1.22 mg/l), in comparison to the reference strain, *B. subtilis* MTCC 441 (17 mg/l).

Similarly, isolate MAJ PSB12 synthesized highest content (3.921 ± 0.17 mg/l) of GA_3 in nutrient broth in comparison to control *B. subtilis* MTCC 441 (3.21 mg/l), whereas minimum concentration of GA_3 (0.123 ± 0.13 mg/l) was produced by MAJ PSB01. Production of GA_3 by isolates KAZ PSB01 (3.417 ± 0.14 mg/l), MAJ PSB11 (2.403 ± 0.35 mg/l) and MAJ PSB 03 (2.203 ± 0.32 mg/l) was also recorded.

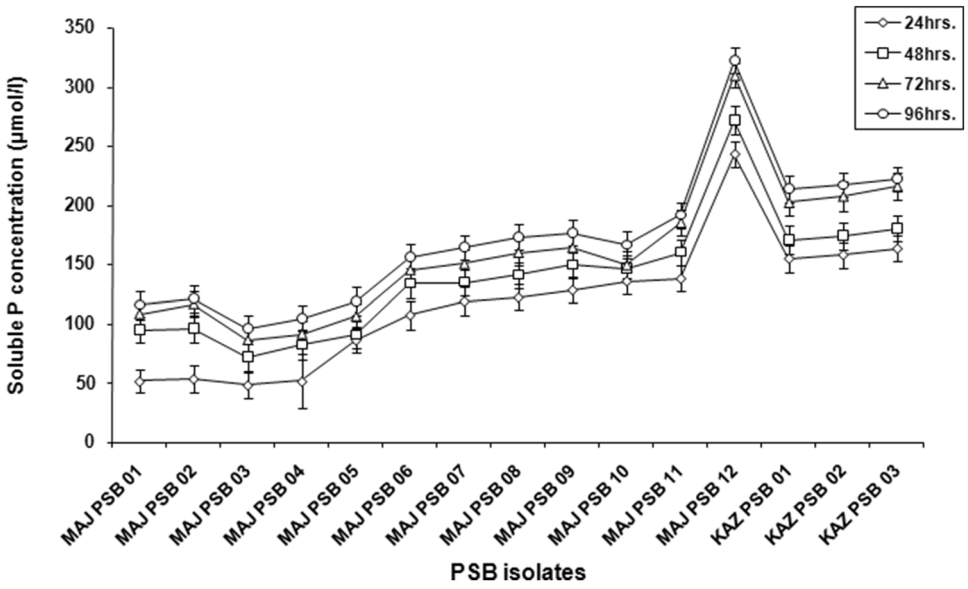

Fig. 2 P solubilization (µmol/l) by PSB isolates at different time intervals

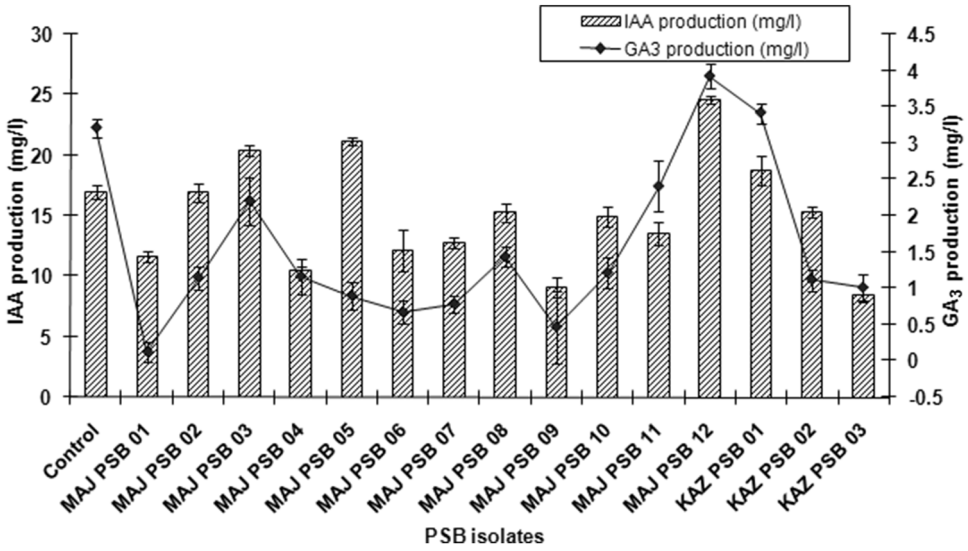

Fig. 3 Productions of IAA and GA_3 (mg/l) by PSB isolates

In the present study, most of the PSB isolates were proficient in PGPR activity by synthesizing plant growth promoting hormones like IAA and GA_3. Soil microorganisms are reported to produce physiologically active IAA which has great effect in plant growth and development. Root exudates are natural source of tryptophan and 80% of the rhizospheric bacteria utilize it as precursor for the production of plant growth hormones [40–42]. Variation in the production of IAA and GA_3 among the isolates depends upon the different metabolic pathway of PSB [40]. GA_3 produced by PSB can influence the growth components of higher plants which can improve the root growth and eventually enhance the nutritional uptake from soil [43].

Identification

Phenotypic study revealed that isolate MAJ PSB12 is rod-shaped, size (length 2.0–3.2 µM × width 0.6–1.0 µM), Gram-positive, aerobic and spore-forming. Based on biochemical characterization the most efficient PSB isolate was positive in hydrolysis of starch and production of urease, catalase, nitrate reductase, phosphatase, methyl red, gelatin liquefaction, indole, and arginine whereas showed negative VP test, citrate, H_2S production and ONPG test. The bacterium, MAJ PSB12 assimilated glucose, arabinose, maltose, mannitol, glycerol, sucrose, and trehalose however did not utilize xylose, adonitol, galactose,

gluconate, lactose, melibiose, rhamnose, ribose, sorbitol and xylitol (Table 1).

A total of 1404 bp of 16S rRNA gene of the strain MAJ PSB12 was amplified and sequenced using their respective primers. The amplified gene has got approximate molecular weight ~1.5 kb as resemblance to the StepUpTM 500 bp DNA ladder (Fig. 1b). Comparison of these test sequences against NCBI GenBank database was performed with BLAST. As per the BLAST result, the 16S rDNA sequence of isolate MAJ PSB12 has 99% sequence similarity having highest score i.e. 2514 bits with *Bacillus firmus* NBRC 15306. Subsequently, a 16S rDNA gene sequence was aligned and phylogenetic tree was constructed, which shows the relationship between the isolate MAJ PSB12 and closely homologous group of bacteria (Fig. 1c). Based on the 16S rDNA homology to *B. firmus,* the isolate MAJ PSB12 was referred to as *Bacillus firmus* strain MAJ PSB12 with NCBI Gene Bank accession number KM068057. The DNA G+C content of strain MAJ PSB12 was determined as 41.0 mol percent. The most efficient P solubilizer was identified as *Bacillus firmus* MAJ PSB12. The 16S rDNA homology study is considered to be a central measure to recognize microbial diversity and identification of novel strains with a considerable variation between species in both length and sequence of the 16S rDNA ITS region [44]. It has already been reported that bacterial species belonging to the genus *Bacillus* produce 2-ketogluconic, oxalic and succinic acids are capable of solubilizing inorganic phosphates [45]. But, the advantage of using native rhizosphere PSB over the bacteria isolated from different localities or environment is an easier adaptation and succession during bio-fertilization process.

Conclusion

Use of bio-fertilizers has now become an immense demand for sustainable agriculture [29]. Eri silkworm rearers of northeastern part of the country are reluctant for application of bio-fertilizers for host plant cultivation due to lack of knowledge or awareness. This may be due to limitation of studies in this particular field of host plant nutrition or management. Successful introduction of biofertilizer practice for silkworm host plant will be a boon towards sustainable sericulture.

The present study concluded that *Bacillus firmus* MAJ PSB12 is efficient in solubilizing the insoluble tri-calcium phosphate by producing multiple organic acids and thereby leading to decrease in the pH of the culture medium. Moreover, *B. firmus* MAJ PSB12 isolated from castor rhizosphere is capable of producing plant growth promoting substances like IAA and GA_3. Evaluation of *in-vivo* efficiency of this bacterium is required for its utilization as biofertilizer to minimize the application of inorganic P and to reduce environmental pollution as well as to promote sustainable castor cultivation for sericulture.

Acknowledgements The authors are grateful to the Director, Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam (India) for providing the facilities and Department of Science and Technology (DST), Govt. of India, New Delhi (India) for providing the fund to conduct the work.

References

1. Woo SM, Lee MK, Hong IS, Poonguzhali S, Sa TM (2010) Isolation and characterization of phosphate solubilizing bacteria from Chinese cabbage. In: 19th world congress of soil science, vol. 1, pp 56–59
2. McLaughlin MJ, Tiller KG, Naidu R, Stevens DP (1996) Review: the behaviour and environmental impact of contaminants in fertilizers. Aus J Soil Res 34:1–54
3. Bolan NS, Adriano DC, Naidu R (2003) Role of phosphorus in immobilization and bioavailability of heavy metals in the soil-plant system. Rev Environ Contam Toxicol 177:1–44
4. He ZL, Zhang MK, Calvert DV, Stoffella PJ, Li YC (2003) Loading of phosphorus in surface runoff in relation to management practices and soil properties. Soil Crop Sci Soc Fla Proc 62:12–20
5. Sharpley AN, Daniel T, Sims T, Lemunyon J, Stevens R, Parry R (2003) Agricultural phosphorous and eutrophication, 2nd edn. Department of Agriculture, Agricultural Research Service, Lincoln, p 149
6. De Souza MJBD, Nair S, Chandramohan D (2000) Phosphate solubilizing bacteria around Indian Peninsula. Ind J Mar Sci 29:48–51
7. Afzal A, Ashraf M, Asad AS, Farooq M (2005) Effect of phosphate solubilizing microorganisms on phosphorus uptake, yield, and yield traits of wheat (*Tritium aestivum*) in rain fed area. Int J Agric Biol 7(2):207–209
8. Afzal A, Ashgari B (2008) *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). Int J Agric Biol 10(1):85–88
9. Ranjan A, Mahalakshmi MR, Sridevi M (2013) Isolation and characterization of phosphate-solubilizing bacterial species from different crop fields of Salem, Tamil Nadu, India. Int J Nutr Pharmacol Neurol Dis 3(1):29–33
10. Widawati S (2011) Diversity and phosphate solubilization by bacteria isolated from laki island coastal ecosystem. Biodiversity 12(1):17–21
11. Reyes I, Bernier L, Simard R, Antoun H (1999) Effect of nitrogen source on solubilization of different organic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. FEMS Microbiol Ecol 28:281–290
12. Kumar A, Kumar A, Devi S, Patil S, Chandani P, Nagi S (2012) Isolation, screening and characterization of bacteria from rhizospheric soils from different plant growth promotion activities: as in vitro study. Recent Res Sci Technol 4(1):1–5
13. Jones DL (1998) Organic acids in the rhizosphere—a critical review. Plant Soil 205(1):25–44
14. Archana G, Buch A, Naresh KG (2012) Pivotal role of organic acid secretion by rhizobacteria in plant growth promotion. In: Satyanarayana T, Johri BN, Prakash A (eds) Microorganisms in sustainable agriculture and biotechnology. Springer, Berlin, p 3553
15. Fankem H, Nwaga D, Deubel A, Merbach W, Etoa FX (2006) Occurence and functioning of phosphate solubilizing

microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. Afr J Biotechnol 5(24):2450–2460
16. Patel D, Parmar P (2013) Isolation and screening of phosphate solubilizing bacteria from sunflower rhizosphere. Glob J Biosci Biotechnol 2(3):438–441
17. Smibert RM, Kreig NR (1981) Generation characterization. In: Gerhard PM, Castillow RN, Nester EW, Wood WA, Kreig NR, Philips GB (eds) Manual of methods for general bacteriology. American Society for Microbiology, Rahway, pp 409–443
18. Holt JG, Krieg NR, Sneath PHA, Staley JT (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams & Wilkins, Baltimore, p 175
19. Edi Premono M, Moawad AM, Vlek PLG (1996) Effect of phosphate- solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. Indones J Crop Sci 11(1996):13–23
20. Islama T, Deoraa A, Hashidokoa Y, Rahmana A, Itoa T, Taharaa S (2007) Isolation and identification of potential phosphate solubilizing bacteria from the rhizoplane of *Oryza sativa* L. *cv. BR29 of Bangladesh*. Z Naturforsch 62(c):103–110
21. Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chem Acta 27:31–36
22. Wohler I (1997) Auxin-indole derivatives in soils determined by a colorimetric method and by high performance liquid chromatography. Microbiol Res 152:399–405
23. Marques APGC, Pires C, Moreira H, Rangel AOSS, Castro PML (2010) Assessment of the plant growth promotion abilities using *Zea mays* as indicator plant. Soil Biol Biochem 42:1229–1235
24. Paleg LG (1965) Physiological effects of gibberellins. Annu Rev Plant Physiol 16:291–322
25. Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 3:208
26. Weisberg WG, Barns SM, Pelletier BA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703
27. Saitou N, Nie M (1987) The Neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
28. Chakraborty A, Hema MR, Rajgopal R, Jain M, Yadav R, Siddalingeshwara KG, Pramod T (2014) Isolation and characterization of potential plant growth promoting rhizobacteria from non-rhizospheric soil. Int J Curr Microbiol App Sci 3(4):432–438
29. Gulati A, Vyas P, Rahi P, Kasana RC (2009) Plant growth-promoting and rhizosphere-competent *Acinetobacter rhizosphaerae* strain BIHB 723 from the cold deserts of the Himalayas. Curr Microbiol 58:371–377
30. Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
31. Raghu K, MacRae IC (1966) Occurrence of phosphate-dissolving microorganisms in the rhizosphere of rice plants and in submerged soils. J Appl Bacteriol 29:582–586
32. Coats VC, Rumpho ME (2014) The rhizosphere microbiota of plant invaders: an overview of recent advances in the microbiomics of invasive plants. Front Microbiol 5:368
33. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tri calcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41
34. Hwangbo H, Park RD, Kim YW, Rim YS, Park KH, Kim TH, Suh JS, Kim KY (2003) 2-Ketogluconic acid production and phosphate solubilization by enterobacter intermedium. Curr Microbiol 47:87–92
35. Asea PEA, Kucey RMN, Stewart JWB (1988) Inorganic phosphate solubilization by two *Penicillum* species in solution culture and soil. Soil Biol Biochem 20:459–464
36. Salih HM, Yahya AI, Rehman AA, Munam BH (1989) Availability of phosphorus in a calcareous soil treated with rock phosphate or super-phosphate or affected by phosphate dissolving fungi. Plant Soil 20:181–185
37. Whitelaw MA, Harden TJ, Helyar KR (1999) Phosphate Solubilization in solution culture by the soil fungus *Penicillum radicum*. Soil Biol Biochem 32:655–665
38. Illmer P, Schinner F (1992) Solubilization of inorganic calcium phosphate—solubilising mechanisms. Soil Biol Biochem 27:257–263
39. Martens DA, Frankenberger WT WT Jr (1994) Assimilation of exogenous 2–14C indole acetic acid and 3–14C tryptophan exposed to the roots of three wheat varieties. Plant Soil 166:281–290
40. Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18
41. Tsavkelova EA, Cherdyntseva TA, Botina SG, Netrusov AI (2007) Bacteria associated with orchid roots and microbial production of auxin. Microbiol Res 162:69–76
42. Leinhos V, Vacek O (1994) Biosynthesis of auxins by phosphate solubilizing rhizobacteria from wheat and rye. Microbiol Res 149:31–35
43. Thimann KV (1972) Physiological actions of auxins. In: Steward FC (ed) Plant physiology. Academic Press, New York, pp 63–123
44. Magray MSUD, Kumar A, Rawat AK, Srivastava S (2011) Identification of *Escherichia coli* through analysis of 16S rRNA and 16S-23S rRNA internal transcribed spacer region sequences. Bioinformation 6(10):370–371
45. Banik S, Dey BK (1983) Phosphate solubilizing potentiality of the microorganisms capable of utilizing aluminium phosphate a sole phosphate source. Zentralbl Mikrobiol 138:17–23